Nitrogen Fixation in Wood in Ground Contact

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1. Introduction

Several authors (Seidler, Aho, Raju and Evans, 1972; Sharp and Millbank, 1973, and Cornaby and Waide, 1973), have reported the presence of nitrogen fixing activity in wood. All suggested that the nitrogen fixing organisms could be of significance in decaying timber as they may provide the fungi concerned with nitrogen.

Sharp (1975) in a preliminary survey of penetration into wood used partially sealed centimetre cubes of several species in ground contact for 30 days. In the present study larger blocks have been sampled at intervals in order to observe the pattern and direction of penetration, whilst centimetre cubes have been cut and examined in the scanning electron microscope to investigate the distribution of the organisms. To ensure that the entry of soil microorganisms was restricted to one face only, the blocks were coated with a silicone rubber sealant, leaving this particular face exposed. The other surfaces remained sterile beneath the sealant, which could be removed, and any cutting from these sterile surfaces exposed the centre of the block without contamination. Levy (1974) reported the action of bacteria in breaking down the pit membranes and penetrating wood in ground contact. It is highly probable that nitrogen fixing species can also penetrate into wood. Several bacterial isolates from the samples described in this paper have been shown to be capable of fixing nitrogen and it has been assumed that the nitrogen fixing organisms are bacteria.

2. Materials and Methods

2.1 Pattern of penetration of bacteria

Blocks of sapwood of Scots pine (*Pinus sylvestris* L.) and beech (*Fagus sylvatica* L.) 50 mm × 25 mm × 15 mm were selected with the growth rings par-

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allel to the 50 mm × 15 mm face. The blocks were sealed, leaving one tangential face exposed, autoclaved and buried in moist soil. Each sample comprised three blocks of each species, the first sampling being at 25 days and subsequently at 14 day intervals. Each sample block was washed in distilled water, dried, and the sealed surfaces wiped with ethanol. The sealant was removed under aseptic conditions. The block was then split, parallel to the unsealed face, into a series of slices and each slice was split into a number of sticks which were examined for nitrogen fixing ability by the acetylene reduction technique (Postgate, 1972), using glass vials of 28 ml. capacity with a gas mixture of 95 % argon and 5 % carbon dioxide. Acetylene was then injected to produce a concentration of 4 %. Ethylene was determined in samples of the gas by vapour phase chromatography. Samples were taken immediately after injection of acetylene, after 8 and 24 hours incubation at 25° C, and the results expressed as nmoles ethylene produced/hr./gm. wood.

2.2 Direction of penetration of bacteria

Blocks of Scots pine sapwood, selected as before, were sealed except for an area 15 mm × 15 mm on one of the tangential, radial or transverse faces of each block. They were autoclaved and buried in soil out of doors. The blocks were recovered at intervals, treated as before and cut transversely with a circular saw, under aseptic conditions. The sawn pieces were then sliced longitudinally and the segments assayed for acetylene reduction.

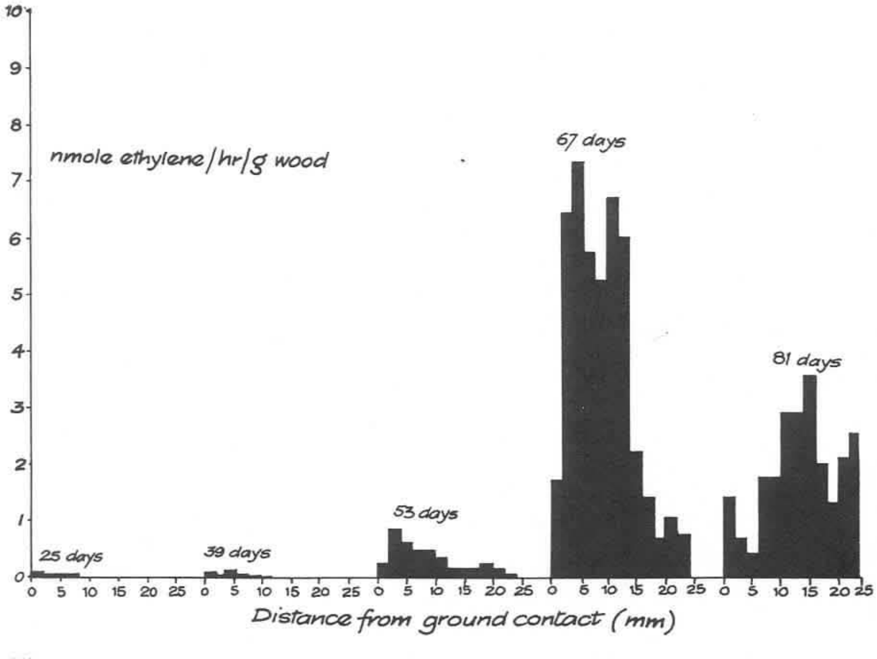

Fig. 1. N_2 (C_2H_2) fixing activity at increasing depths in Scots pine sapwood blocks, tangential face only exposed to ground contact.

2.3 Distribution of bacteria

Oriented centimetre cubes of sapwood of Scots pine and beech were sealed, leaving a tangential face exposed. They were buried in soil, removed at 10 day intervals, sampled as above, split into 1 mm thick radial longitudinal sections, air dried overnight, coated with gold and examined in a Cambridge scanning electron microscope.

3. Results

3.1 Pattern of penetration of bacteria

The results are expressed as histograms (Fig. 1), which indicate the progressive penetration of organisms exhibiting $N_2(C_2H_2)$ fixing activity into the Scots pine blocks from the exposed face in a radial direction, with a peak of activity at 67 days. No activity was found in any of the beech blocks sampled.

3.2 Direction of penetration of bacteria

Only one block per sealing arrangement was analysed at each sampling time, which limits the reliability of the data, though trends were revealed. The blocks with part of the tangential face exposed (Fig. 2)

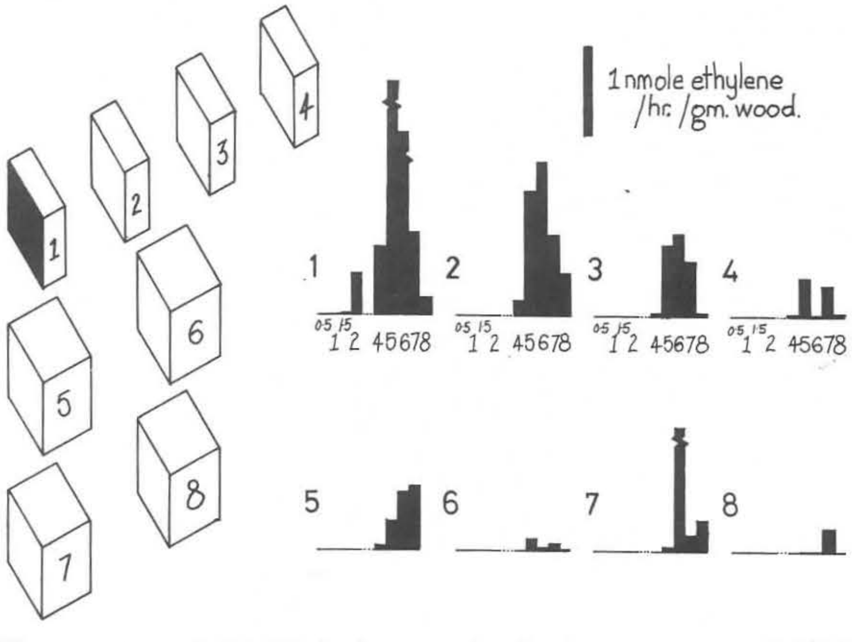

Fig. 2. $N_2(C_2H_2)$ fixing activity in segments of a Scots pine sapwood block, with only the tangential face of segment 1 exposed to ground contact. Sampled at monthly intervals. The 5 and 6 month samples of segment 1 are reduced to 1/4. The 6 month sample of segment 7 is reduced to 1/2.

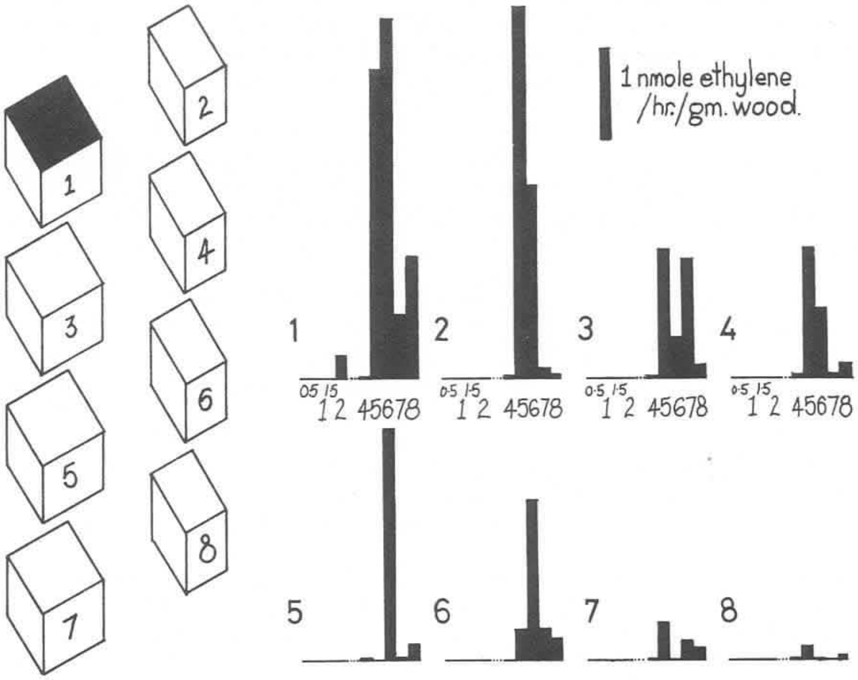

Fig. 3. $N_2(C_2H_2)$ fixing activity in segments of a Scots pine sapwood block, with only the transverse face of segment 1 exposed to ground contact. Sampled at monthly intervals.

showed a similar pattern to the previous experiment, a progressive penetration in a radial direction, together with some penetration in a longitudinal direction. The highest activity was at five months. Penetration from the transverse exposed face (Fig. 3) was both radial and longitudinal, with the highest activity again at five months. When the radial face was exposed (Fig. 4) the colonisation of the exposed face (Segment 1) appeared at $1^1/_2$ months, earlier than in the other sealing arrangements. Higher activity was found in segment 3 than in segment 2, which indicated that nitrogen fixing bacteria were able to penetrate more easily in a radial than in a tangential direction.

3.3 Distribution of bacteria

Although the SEM is limited in its use as an instrument for assessing the distribution of nitrogen fixing bacteria because it is not possible to differentiate between these and non-fixing bacteria when viewed on the specimen, a qualitative examination of the Scots pine cubes revealed the presence, at 50 and 60 days, of bacteria (particularly associated with

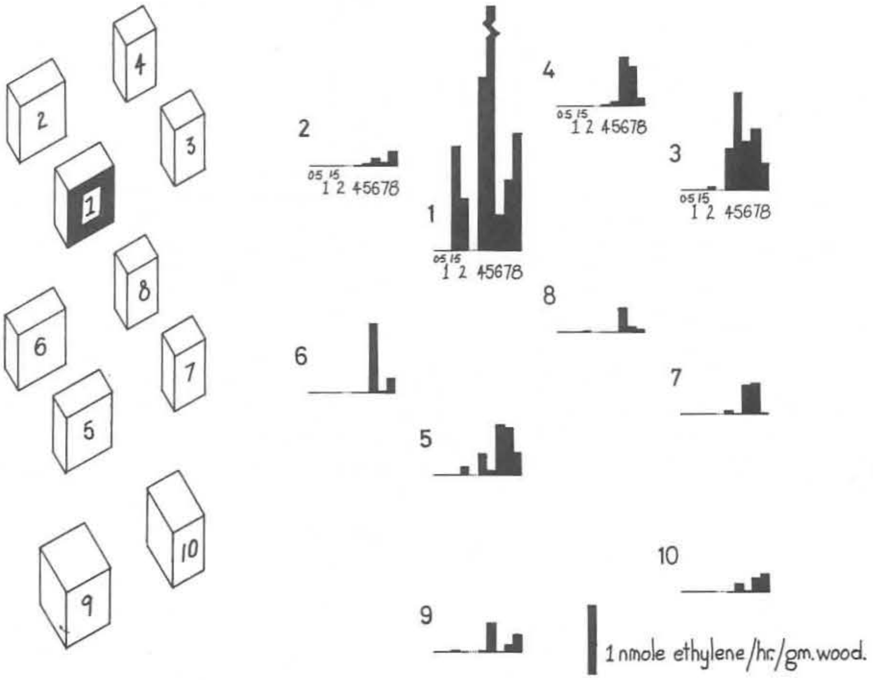

Fig. 4. $N_2(C_2H_2)$ fixing activity in segments of a Scots pine sapwood block, with only the radial face of segment 1 exposed to ground contact. Sampled at monthly intervals. The 5 month sample of segment 1 is reduced to $^1/_2$.

the rays) and fungal hyphae. No bacteria were observed in the beech cubes.

4. Discussion

The results demonstrated the penetration of $N_2(C_2H_2)$ fixing organisms into partially sealed Scots pine blocks and indicated that the ease of penetration in the radial direction was greater than in the tangential direction. The levels of activity as illustrated by the increase in activity at 67 days in controlled conditions and 5 months when exposed outside, may represent an increase in numbers or an increase in individual activity caused by change in the condition of the wood. Further work will be necessary to determine the cause of this peak of activity.

The absence of $N_2(C_2H_2)$ activity from beech blocks may be explained by the abnormal behaviour of beech rays (LEVY, 1965), where the substances in the cells appear to prevent the penetration of fungi as well as nitrogen fixing bacteria.

The presence of bacteria clustered around the rays is only a qualitative result since it is based on observations of some 25 rays per section. This is only a small percentage of the total present, but its consistency suggests a possible distribution.

5. Conclusions

Nitrogen fixing (C_2H_2) bacteria are capable of penetrating Scots pine sapwood in ground contact, primarily along the rays. The significance of their presence is yet to be determined, but their presence alone contributes to the ecology of the wood, and their ability to fix nitrogen could make an even more significant contribution.

6. Summary

The penetration of nitrogen fixing bacteria into partially sealed oriented blocks of sapwood of Scots pine and beech in ground contact was investigated by the acetylene reduction technique, and by observation in the SEM. The results indicate that the bacteria primarily penetrate along the rays in Scots pine but do not penetrate into beech.

Zusammenfassung

Stickstoff-Fixierung in Holz bei Bodenkontakt

Das Eindringen Stickstoff-fixierender Bakterien in Kiefern- und Buchensplintholz wurde unter Anwendung der Acetylen-Reduktionsmethode sowie anhand rasterelektronenmikroskopischer Beobachtungen verfolgt. Ein Teil der orientierten Außenflächen der Proben war hierzu versiegelt worden. Die Ergebnisse zeigen, daß Bakterien in Kiefernholz über die Holzstrahlen, nicht dagegen in Buchenholz einzudringen vermögen.

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